Annocherin and (2,4)-*cis*- and *trans*-Annocherinones, Monotetrahydrofuran Annonaceous Acetogenins with a C-7 Carbonyl Group from *Annona cherimolia* Seeds

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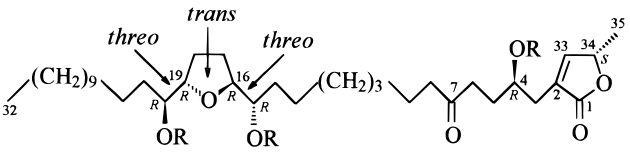
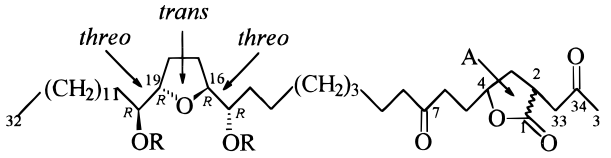
The new cytotoxic monotetrahydrofuran Annonaceous acetogenins, annocherin (**1**) and a mixture of (2,4)-*cis*- and *trans*-annocherinones (**2** and **3**), were isolated from the bioactive methanolic extract of *Annona cherimolia* seeds. Compounds **1**−**3** each possess an unusual 7-carbonyl group. Their structures were established on the basis of chemical and spectral evidence. Compounds **1**−**3** showed significant toxicity in the brine shrimp lethality test and cytotoxicity for six human solid tumor cell lines, with selectivity for the renal cell line (A-498) at potencies equivalent to Adriamycin.

Annona cherimolia Mill. (Annonaceae) is a tropical tree native to South America (Peru)[1] and is cultivated worldwide for its edible fruits ("cherimoya"). The plant has been used in traditional medicine as an insecticide and a parasiticide.[2] Previous bioactivity-directed isolation has led to the discovery of approximately 21 bioactive acetogenins from the seeds and roots.[3−10] In our investigation of this plant, we have isolated novel acetogenins from an ethanol extract of *Annona cherimolia* seeds, and the compounds are annocherin (**1**) and a mixture of (2,4)-*cis*- and *trans*-annocherinones (**2**, **3**). Compounds **1**−**3** are monotetrahydrofuran (mono-THF) acetogenins with a new structural feature in that a carbonyl group is located at C-7 in the hydrocarbon chain.



Compound	R
1	H
1a	Ac
1b	TMSi
1r	(*R*)-MTPA
1s	(*S*)-MTPA



Compound	A	R
2	*cis*	H
2a	*cis*	Ac
2b	*cis*	TMSi
2r	*cis*	(*R*)-MTPA
2s	*cis*	(*S*)-MTPA
3	*trans*	H
3a	*trans*	Ac
3b	*trans*	TMSi
3r	*trans*	(*R*)-MTPA
3s	*trans*	(*S*)-MTPA

Results and Discussion

The most active fraction, F005, was obtained from the ethanol extract (F001) of the seeds by partition between dichloromethane (F003) and water and subsequent partition of F003 between hexane and 90% aqueous methanol (F005) under the direction of the brine shrimp lethality test (BST).[11,12] F005 was submitted to successive Si gel and HPLC chromatographic steps, monitoring with the BST and leading to the isolation of compounds **1**−**3**.

Compound **1**, $[\alpha]^{20}_{D}$ +1.1°(*c* 0.01, CH_2Cl_2), was obtained as a white powder. The HRFABMS gave a $[M + Na]^+$ ion at *m*/*z* 617.4413 (calcd 617.4393) corresponding to the formula, $C_{35}H_{62}O_7Na$. The IR spectrum suggested the presence of OH groups, with a peak at 3437 cm^{-1}, and the presence of an α,*β*-unsaturated *γ*-lactone, with a peak at 1751 cm^{-1}. The NMR spectra of **1** showed ^1H NMR resonances at *δ* 7.18 (H-33), 5.06 (H-34), 2.38 (H-3a), 2.50 (H-3b), 3.81 (H-4), and 1.43 (H-35) (Table 1) and six ^{13}C NMR resonances at *δ* 174.65 (C-1), 151.92 (C-33), 131.14 (C-2), 78.03 (C-34), 69.86 (C-4), and 19.11 (C-35) (Table 1). These are characteristic spectral features of α,*β*-unsaturated *γ*-lactones with a C-4 hydroxyl group, as is common in many of the Annonaceous acetogenins.[13,14]

The successive losses of H_2O (18 u) from the $[MH]^+$ of **1** in the FABMS (*m*/*z* 595, 577, 559, and 541) demonstrated the existence of three OH groups, and these were confirmed by the formation of triacetate (**1a**) and tri-TMSi derivatives (**1b**). Mass spectrometric analysis of the triacetate (**1a**) and tri-TMSi derivatives (**1b**) demonstrated that the three OH groups were located at C-4, C-15, and C-20, as shown in

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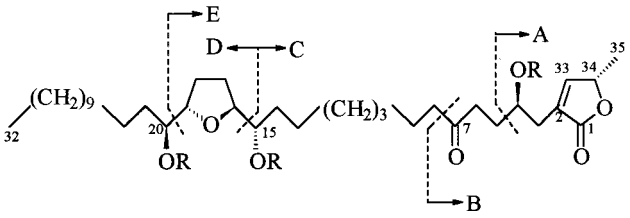
Table 1. ^{1}H NMR Spectral Data of **1** and **1a**, and ^{13}C NMR Spectral Data of **1** ($CDCl_3$, δ)

	^{1}H NMR (300 MHz)		^{13}C NMR (75.5 MHz)
position	**1**	**1a**	**1**
1			174.65
2			131.14
3a	2.38 ddt (15.0, 8.5, 1.8)	2.37 ddt (15.0, 8.5, 1.8)	33.46
3b	2.50 dt (15.5, 1.8)	2.54 dt (15.5, 1.8)	
4	3.81 m	5.10 m	69.86
5	1.26 br s	1.26 br s	23.64[a]
6	2.42 t (6.3)	2.40 t (1.8)	42.60[b]
7			211.38
8	2.42 t (6.3)	2.40 t (1.8)	42.70[b]
9	1.26 br s	1.26 br s	23.79[a]
10−14	1.26 br s	1.26 br s	22.69−37.12
15	3.41 m	4.85 m	74.10[c]
16	3.81 m	3.96 m	82.68[d]
17a	1.70 m	1.67 m	28.75
17b	1.99 m	1.95 m	
18a	1.70 m	1.67 m	28.75
18b	1.99 m	1.95 m	
19	3.81 m	3.96 m	82.54[d]
20	3.41 m	4.85 m	73.80[c]
21−31	1.26 br s	1.26 br s	22.69−37.12
32	0.88 t (6.9)	0.88 t (6.9)	14.13
33	7.18 q (1.5)	7.08 q (1.5)	151.92
34	5.06 qq (7.0, 1.5)	5.01 qq (8.7, 1.8)	78.03
35	1.43 d (6.9)	1.40 d (6.9)	19.11
OAc-4		2.02 s	
OAc-15		2.07 s	
OAc-20		2.07 s	

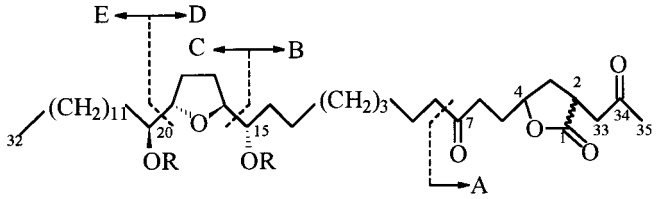
[a−d] Assignments may be reversed in each column.



R	$MH^+/M(Ac)_3^+$ /$M(TMSi)_3^+$	A	B	C	D	E
H	595, 577(a), 559(a), 541(a)	141, 123(a)	197, 179(a)	325, 307(a), 289(a)	269, 251(a)	395, 377(a), 359(a)
Ac	720, 660(b), 600(b), 540(b)	183, 123(b)	239, 179(b)	409, 349(b), 289(b)	311, 251(b)	479, 419(b), 359(b)
TMSi	810, 720(c), 630(c), 540(c)	213, 123(c)	269, 179(c)	469, 379(c), 289(c)	341, 251(c)	539, 449(c), 359(c)

Figure 1. Diagnostic EIMS fragmentations (*m*/*z*) of **1** and its triacetate (**1a**) and tri-TMSi (**1b**) derivatives; (a) loss of H_2O (18 u); (b) loss of HOAc (60 u); (c) loss of TMSiOH (90 u).

Figure 1. In the ^{1}H NMR spectrum, a three-proton multiplet was visible at δ 3.81 due to the two oxymethine protons of the tetrahydrofuran (THF) ring as well as the carbinol methine proton at C-4. In the ^{13}C NMR spectrum, the oxymethine carbons of the THF ring appeared at δ 82.68 (C-16) and 82.54 (C-19). Also in the ^{1}H NMR spectrum, a two-proton multiplet was seen at δ 3.41 due to two carbinol methine protons, indicating the presence of a mono-THF ring with two flanking hydroxyl groups.[13,14] Determination of the relative stereochemistry around the mono-THF moiety was accomplished by Born's technique[15] and by comparison with reported spectral data of a series of synthetic mono-THF diol compounds of known relative configuration.[16] The arrangement was assigned as *threo* between C-15/C-16 and C-19/C-20 because of the proton signals at δ 3.41 for H-15 and H-20 and carbon signals at δ 74.10 for C-15 and 73.80 for C-20; the *trans* relationship between C-16/C-19 was determined by comparison with reported spectral data of model compounds,[15,16] suggesting that the relative configuration for these four chiral carbons was *threo*/*trans*/*threo*. The presence of a keto group in the long aliphatic chain was evident from the carbonyl carbon shift at δ 211.38 and the adjacent methylene carbon shifts at δ 42.6 and 42.7 in the ^{13}C NMR spectrum. In the ^{1}H NMR spectrum, the protons of the above methylene groups appeared at δ 2.37−2.52 together with the C-3 protons. The carbonyl position was suggested to be at C-7 based on a peak at *m*/*z* 197 in the EIMS of **1**. This assignment was predicted on the assumption that cleavage was between C-7 and C-8, assuming that the oxygen was included in

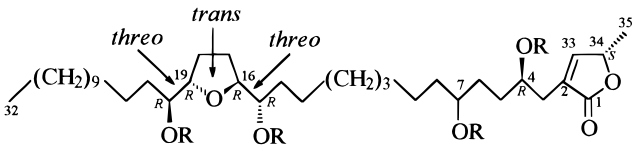


R	$MH^+/M(Ac)_2^+$ /$M(TMSi)_2^+$	A	B	C	D	E
H	595 577(a), 559(a)	197	325, 307(a)	269, 251(a)	395, 377(a)	199, 181(a)
Ac	678, 618(b), 558(b)	197	367, 307(b)	311, 251(b)	437, 377(b)	241, 181(b)
TMSi	738, 648(c), 558(c)	197	397, 307(c)	341, 251(c)	467, 377(c)	271, 181(c)

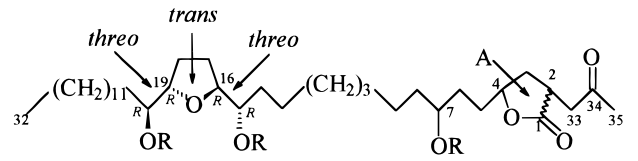
Figure 2. Diagnostic EIMS fragmentations (*m*/*z*) of **2** and **3** and their diacetate and di-TMSi derivatives; (a) loss of H_2O (18 u); (b) loss of HOAc (60 u); (c) loss of TMSiOH (90 u).

the fragment ion (Figure 1). Confirmation of the structure was achieved by reduction of **1** with $NaBH_4$, which yielded a racemic alcohol (**4**).[17] The carbonyl position was determined from the EIMS fragment ion at *m*/*z* 343, which can be explained by a C-7/C-8 cleavage of the TMSi derivative (**4a**) of the alcohol derivative. Compound **1** is a C_{35} mono-THF acetogenin with a novel 7-carbonyl group; it was named annocherin.

Compounds **2** and **3**, $[\alpha]^{20}_D$ −1.3°(*c* 0.005, CH_2Cl_2), were obtained in a mixture as a white powder. HRFABMS gave $[M + Na]^+$ ions at *m*/*z* 617.4402 (calcd 617.4393) corresponding to the formula, $C_{35}H_{62}O_7Na$. The IR spectrum showed a strong absorption at 1703 cm^{-1} for keto carbonyls. The ^1H and ^{13}C NMR spectra of **2** and **3** clearly indicated the presence of a ketolactone moiety.[14,18−20] In the ^1H NMR spectrum, the resonances at δ 4.53 and 4.39, with a combined integration for one proton, were assigned to H-4 and suggested the presence of the (2,4)-*cis*- and *trans*-diastereoisomers at the ketolactone ring moiety, as is typical with the ketolactones.[20] Sequential losses of two molecules of H_2O from the $[MH]^+$ in the FABMS indicated the presence of two hydroxyl groups in **2** and **3**, which were supported by a broad hydroxyl absorption at 3483 cm^{-1} in the IR spectrum. In the ^{13}C NMR spectrum of **2** and **3**, signal pairs at δ 178.24 and 178.72, 35.38 and 33.49, 79.38 and 78.87, and 205.58 and 205.51 were assigned to C-1, C-2, C-4, and C-34, respectively, and confirmed the presence of *cis*- and *trans*-isomers. The placements of the mono-THF ring system at C-16 to C-19 and the two flanking OH groups at C-15 and C-20 were suggested based on the EIMS fragmentation pattern of the diacetate (**2a**) and di-TMSi derivatives (**2b**). The assignments of the relative stereochemistries around the mono-THF rings were determined using the methodologies of Hoye et al.[21,22] and Born et al.,[15] and by comparison with several similar acetogenins. In addition, the paper by Fujimoto et al.[23] describes model mono-THF analogues, with flanking hydroxyls, having all possible relative stereochemistries; **2** and **3** matched very well with the *threo*/*trans*/*threo* model. The presence of a keto group in the long aliphatic chain was evident from the carbonyl ^{13}C NMR shift at δ 211.30 and the adjacent methylene carbon shifts at δ 42.51 and 42.62. In the ^1H NMR spectrum, the protons of the above methylene groups appeared at δ 2.37−2.44. The carbonyl position was suggested to be at C-7, as in **1**, based on a peak at *m*/*z* 197 in the EIMS of **2** and **3** (Figure 2). Confirmation of the structure was achieved by reduction of the mixture **2** and **3** with $NaBH_4$, which yielded the racemic alcohols (**5** and **6**). The location of the carbonyl group was determined from the EIMS fragment ion at *m*/*z* 271, which can be explained by a C-7/C-8 cleavage of the TMSi derivatives (**5a** and **6a**) of the mixture, **5** and **6**. Compounds **2** and **3** are, thus, the ketolactones of **1**, and were named (2,4)-*cis*- and *trans*-annocherinones.



Compound	R
4	H
4a	TMSi



Compound	A	R
5	*cis*	H
5a	*cis*	TMSi
6	*trans*	H
6a	*trans*	TMSi

Rieser et al. have reported the determination of the absolute configurations of stereogenic carbinol carbons in several Annonaceous acetogenins using Mosher ester methodology.[24−26] Thus, the (*S*)- and (*R*)-methoxyfluoromethylphenylacetic acid (MTPA) esters (Mosher esters) of **1** and the mixture of **2** and **3** were prepared and numbered **1s**, **1r**, **2s**, **2r**, **3s**, and **3r**, respectively. COSY ^1H NMR analysis of these derivatives was then performed. The $\Delta\delta_H$ values for H-33 and H-34 in **1r** and **1s** at 0.25 and 0.06 suggested that **1** has the 4*R*,34*S* arrangement. All of the C_{35}, OH-4 Annonaceous acetogenins, so far, are 4*R* and

Table 2. ^{1}H NMR Spectral Data of **2**, **2a**, **3**, and **3a** and ^{13}C NMR Spectral Data of **2** and **3** ($CDCl_3$, δ)

	^{1}H NMR (300 MHz)				^{13}C NMR (75.5 MHz)	
position	**2**	**2a**	**3**	**3a**	**2**	**3**
1					178.24	178.72
2	3.06 m	3.05 m	3.01 m	3.01 m	35.38	33.49
3a	1.40 m	1.45 m	1.99 m	2.02 m	25.60−31.91	25.60−31.91
3b	2.61 ddd (12.3, 9.4, 5.6)	2.63 ddd (12.3, 9.4, 5.6)	2.22 ddd (12.9, 9.6, 3.4)	2.24 ddd (12.9, 9.6, 3.4)		
4	4.39 dddd (12.3, 9.5, 4.3, 3.0)	4.39 dddd (12.3, 9.5, 4.3, 3.0)	4.53 dddd (8.3, 8.2, 5.7, 3.2)	4.54 dddd (8.3, 8.2, 5.7, 3.2)	79.38	78.87
5	1.26 br s	1.25 br s	1.26 br s	1.25 br s	36.72	35.47
6	2.40 t (14.1)	2.39 t (14.3)	2.40 t (14.1)	2.39 t (14.3)	42.51[a]	42.51[b]
7					211.30	211.30
8	2.40 t (14.1)	2.39 t (14.3)	2.40 t (14.1)	2.39 t (14.3)	42.62[a]	42.62[b]
9−14	1.26 br s	1.26 br s	1.26 br s	1.26 br s	25.60−31.91	25.690−31.91
15	3.42 m	4.85 m	3.42 m	4.85 m	74.04[c]	74.03
16	3.81 m	3.97 m	3.81 m	3.97 m	82.61	82.61
17a	1.66 m	1.67 m	1.66 m	1.67 m	28.72	28.72
17b	1.99 m	1.96 m	1.99 m	1.96 m		
18a	1.66 m	1.67 m	1.66 m	1.67 m	28.72	28.72
18b	1.99 m	1.96 m	1.99 m	1.96 m		
19	3.81 m	3.97 m	3.81 m	3.97 m	82.61	82.61
20	3.42 m	4.85 m	3.42 m	4.85 m	74.03[c]	74.03
21−31	1.26 br s	1.26 br s	1.26 br s	1.26 br s	25.60−31.91	25.60−31.91
32	0.88 t (6.7)	0.88 t (6.8)	0.88 t (6.7)	0.88 t (6.8)	14.11	14.11
33a	2.63 dd (18.5, 9.0)	2.59 dd (19.0, 9.0)	2.67 dd (18.5, 9.0)	2.63 dd (19.0, 9.0)	43.83	44.28
33b	3.15 dd (10.1, 1.5)	3.15 dd (10.0, 1.5)	3.03 dd (7.3, 4.0)	3.03 dd (7.1, 3.7)		
34					205.58	205.51
35	2.20 s	2.20 s	2.20 s	2.20 s	25.18	25.58
OAc-15		2.07 s		2.07 s		
OAc-20		2.07 s		2.07 s		

[a−c] Assignments may be reversed in each column.

Table 3. ^{1}H NMR (500 MHz, $CDCl_3$) Chemical Shifts for the Determination of the Absolute Configurations at C-4, C-15, and C-20 of the Tri-(*S*)- and -(*R*)-MTPA Esters of **1**

MTPA ester	5-Hab	4-H	3-Hab	33-H	34-H	35-H	14-Hab	15-H	16-H	19-H	20-H	21-Hab
1r	1.57 1.67	5.38	2.62 2.66	6.98	4.93	1.32	1.39 1.57[b]	5.04	4.01	4.01	5.04	1.39 1.57
1s	1.64 1.68	5.31	2.50 2.60	6.73	4.87	1.26	1.57 1.65[d]	4.95	3.92	3.92	4.95	1.57 1.65
$^{\Delta\delta}$**1s−1r**	+0.07 +0.01	−0.07	−0.12 -0.06	−0.25	−0.06	−0.06	+0.18 +0.08	−0.09	−0.09	−0.09	−0.09	+0.18 +0.08
	absolute configuration							C-4 *R*; C-15 *R*; C-20 *R*				

Table 4. ^{1}H NMR (500 MHz, $CDCl_3$) Chemical Shifts for the Determination of the Absolute Configurations at C-15 and C-20 of the Di-(*S*)- and -(*R*)-MTPA Esters of **2** and **3**

MTPA ester	H-14a	H-14b	H-15	H-16	H-17a	H-17b	H-18a	H-18b	H-19	H-20	H-21a	H-21b
2r	1.57	1.49	5.02	4.00	1.56	1.91	1.56	1.91	4.00	5.02	1.52	1.49
2s	1.58	1.55	4.97	3.93	1.39	1.65	1.39	1.65	3.93	4.97	1.58	1.55
$^{\Delta\delta}$**2s−2r**	+0.01	+0.06	−0.05	−0.07	−0.17	−0.26	−0.17	−0.26	−0.07	−0.05	+0.06	+0.06
3r	1.57	1.49	5.02	4.00	1.56	1.91	1.56	1.91	4.00	5.02	1.52	1.49
3s	1.58	1.55	4.97	3.93	1.39	1.65	1.39	1.65	3.93	4.97	1.58	1.55
$^{\Delta\delta}$**3s−3r**	+0.01	+0.06	−0.05	−0.07	−0.17	−0.26	−0.17	−0.26	−0.07	−0.05	+0.06	+0.06
	absolute configuration								C-15 *R*; C-20 *R*			

34*S*. Similarly, the Mosher ester data (Tables 3 and 4) allowed the absolute stereochemical assignments of the carbinol carbons adjacent to the mono-THF rings as C-15*R* and C-20*R* and, consequently, C-16*R* and C-19*R*, in **1**−**3**.

Bioactivity data obtained with **1** and the mixture of **2** and **3** are summarized in Table 5. These compounds are very active in the BST;[11,12] the isolated acetogenins showed significant selective cytotoxicities against A-498 (renal carcinoma)[27] in our seven-day human solid tumor cell *in vitro* tests. This selective cytotoxic activity is comparable to that of the positive control compound, Adriamycin (doxorubicin), which, itself, is more generally cytotoxic. Compound **4** and the mixture of **5** and **6** were less active than compound **1** and the mixture of **2** and **3** against the A-498 (renal carcinoma) cell line, but were about 1000 to 10 000 times more potent than those against the HT-29 (colon adenocarcinoma)[28] and MIA PaCa-2 (pancreatic carcinoma)[30] cell lines. From these data, it can be suggested that the carbonyl at C-7 decreases activity for the HT-29 and MIA PaCa-2 cell lines but augments the potency toward the A-498 cell line. The acetogenins exert their biological effects through inhibition of mitochondrial electron transport (complex I) and the inhibition of the plasma membrane NADH oxidase of cancer cells, and the resulting depletion of ATP thwarts ATP-dependent resistance mechanisms as discussed in our recent reviews.[32,33]

Table 5. Brine Shrimp Lethality (BST) and Cytotoxicity against Human Solid Tumor Cell Lines for **1** and the Mixture of **2** and **3**

		human cancer cell line (ED_{50}, μg/mL)					
compound	BST[a] LC_{50} (μg/mL)	A-549[b]	MCF-7[c]	HT-29[d]	A-498[e]	PC3 [f]	MIA PaCa-2 [g]
1	1.37×10^{-1}	1.67	3.08×10^{-1}	3.54	2.20×10^{-2}	8.62×10^{-1}	2.88
2 and **3**	6.67×10^{-1}	1.55	1.55	1.59	3.16×10^{-2}	4.50	5.15
4	1.25×10^{-2}	1.63	1.1×10^{-2}	1.0×10^{-3}	2.96	1.32	1×10^{-3}
5 and **6**	2.01×10^{-2}	5.51×10^{-1}	1.40	2.81×10^{-3}	1.96	1.05	6.0×10^{-4}
adriamycin[h]	not tested	4.30×10^{-3}	1.29×10^{-1}	1.68×10^{-2}	1.56×10^{-2}	4.99×10^{-2}	1.01×10^{-3}

[a] Brine shrimp test.[11,12] [b] Lung carcinoma.[26] [c] Breast carcinoma.[27] [d] Colon adenocarcinoma.[28] [e] Kidney carcinoma.[26] [f] Prostate adenocarcinoma.[29] [g] Pancreatic carcinoma.[30] [h] Positive control standard.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco micromelting point apparatus and were uncorrected. Optical rotations were taken on a JASCO DIP-370 digital polarimeter. IR spectra were measured on a JASCO FT/IR 300E spectrophotometer. UV spectra were obtained on a Shimadzu UV-1601PC spectrophotometer. ^{1}H, ^{13}C, and COSY NMR spectra were recorded on Varian VXR300S or 500S spectrometers in $CDCl_3$ using TMS as an internal standard. LRFABMS and HRFABMS data were collected on a JEOL JMS−HX110 spectrometer. EIMS were recorded on a Quattro spectrometer. For TLC, Si gel 60 F_{254} (EM 5717) glass plates (0.25 mm) were used and visualized by spraying with 5% phosphomolybdic acid in MeOH and heating. HPLC was performed on a Waters 600 apparatus equipped with a Waters 486 UV detector at 225 nm using the Autochrowin software system (Young Su Scientific Co., Seoul, Korea). μBondapak C_{18} columns (19 × 300 mm and 7.8 × 300 mm) were used for preparative separations.

Plant Material. The seeds of *Annona cherimolia* were obtained in 1996 from fruits grown commercially in plantations in southern California and purchased from Hurov Botanicals and Seeds located in Chula Vista, California.

Bioassays. The extracts, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp larvae (BST). Seven-day *in vitro* MTT cytotoxicity tests against human tumor cell lines were carried out at the Cell Culture Laboratory, Purdue Cancer Center, using standard protocols for A-549 (human lung carcinoma),[27] MCF-7 (human breast carcinoma),[28] HT-29 (human colon adenocarcinoma),[29] A-498 (human kidney carcinoma),[27] PC-3 (human prostate adenocarcinoma),[30] and MIA PaCa-2 (human pancreatic carcinoma)[31] with Adriamycin as a positive control.

Extraction and Isolation. The dried seeds of *Annona cherimolia* (8 kg) were milled, extracted with EtOH, and partitioned, as described above, to obtain fraction F005 (250 g), which was then subjected to open column chromatography over Si gel (2.8 kg) eluted with a gradient of hexane−$CHCl_3$−MeOH. Fractions (F-1 to F-17) were collected and pooled according to their similar TLC patterns. The BST active pool F-12 was further resolved on another Si gel (1.5 kg) open column, eluted with a gradient of hexane−$CHCl_3$−MeOH. Fractions (A−N) were collected into 13 pools on the basis of similar TLC patterns. Further purification of the most bioactive BST fractions was carried out by HPLC eluted with 85% MeCN in H_2O to afford compounds **1** and the mixture of **2** and **3**.

Annocherin (1): white powder (40 mg); mp 80.2−81.5 °C; $[\alpha]^{22}_D$ +1.1° (*c* 0.01, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 227 (3.4) nm; IR (film) ν_{max} 3437, 2920, 2850, 1751, 1707, 1468, 1414, 1377, 1319, 1070 cm^{-1};^{1}H (300 MHz, $CDCl_3$) and ^{13}C (75.5 MHz, $CDCl_3$) NMR, see Table 1; FABMS *m/z* 595 $[MH]^+$, 577 $[MH - H_2O]^+$, 559 $[MH - 2H_2O]^+$, 541 $[MH - 3H_2O]^+$; EIMS *m/z*, see Figure 1; HRFABMS *m/z* $[M + Na]^+$ 617.4413 for $C_{37}H_{68}O_7$ (calcd 617.4393).

Annocherin triacetate (1a): treatment of **1** (2 mg) with anhydrous pyridine and Ac_2O (at room temperature overnight) and subsequent workup gave **1a**; ^{1}H NMR (300 MHz, $CDCl_3$), see Table 1; EIMS *m/z*, see Figure 1.

Annocherin tri-TMSi derivative (1b): approximately 10 μg of compound **1** was treated with 0.2 μL pyridine and 2 μL of *N*,*O*-*bis*-(trimethylsilyl)acetamide for 5 h to give **1b**; EIMS *m/z*, see Figure 1.

(2,4)-*cis*- and *trans*-Annocherinones (2 and 3): white powder (40 mg); mp 76.1−78.0 °C; $[\alpha]^{22}_D$ −1.3° (*c* 0.005, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 205.40 (3.6) nm; IR (film) ν_{max} 3483, 2918, 2848, 1768, 1703, 1465, 1415, 1375, 1319, 1072 cm^{-1}; ^{1}H (300 MHz, $CDCl_3$) and ^{13}C (75.5 MHz, $CDCl_3$) NMR, see Table 1; FABMS *m/z* 595 $[MH]^+$, 577 $[MH - H_2O]^+$, 559 $[MH - 2H_2O]^+$; EIMS *m/z*, see Figure 2; HRFABMS *m/z* $[M + Na]^+$ 617.4402 for $C_{35}H_{64}O_7$ (calcd 617.4393).

(2,4)-*cis*- and *trans*-Annocherinone diacetates (2a and 3a): treatment of the mixture of **2** and **3** (2 mg) with anhydrous pyridine and Ac_2O (at room temperature overnight) and subsequent workup gave a mixture of **2a** and **3a**; ^{1}H NMR (300 MHz, $CDCl_3$), see Table 2; EIMS, see Figure 2.

(2,4)-*cis*- and *trans*-Annocherinone di-TMSi derivatives (2b and 3b): approximately 10 μg of the mixture of **2** and **3** was treated with 0.2 μL pyridine and 2 μL of *N*,*O*-*bis*-(trimethylsilyl)acetamide for 5 h to give a mixture of **2b** and **3b**; EIMS, see Figure 2.

Preparation of Mosher Esters. A previously described method was used.[24−26] To each of 1 mg of **1** and the mixture of **2** and **3** in 0.5 mL of CH_2Cl_2, were added sequentially 0.2 mL pyridine, 0.5 mg 4-(dimethylamino)-pyridine, and 12 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl (*R*-MTPA) chloride, separately. The mixture was left at room temperature overnight and purified over a micro-column (0.6 × 6 cm) of Si gel (230−400 mesh) eluted with 3−4 mL of hexane−CH_2Cl_2 (1:2); the eluate was dried, CH_2Cl_2 (5 mL) was added, and the CH_2Cl_2 was washed using 1% $NaHCO_3$ (5 mL × 3) and H_2O (5 mL × 2); the washed eluate was dried *in vacuo* to give the *S* Mosher esters of **1** and the mixture of **2** and **3**, respectively. Using (*S*)-MTPA chloride afforded the *R* Mosher esters. Their pertinent ^{1}H NMR chemical shifts are given in Tables 3 and 4.

Reduction. To each 2.5 mg of **1** and the mixture of **2** and **3** were sequentially added 0.5 mL of MeOH, 0.05 mL of THF, and 0.025 mL of $NaBH_4$ solution (0.5 M solution in 2-methoxyethyl ether) on an ice-water bath. The mixture was stirred at 0 °C for 10 min and 1 h at room temperature. CH_2Cl_2 was added to form two distinct layers, and the CH_2Cl_2 layer was dried *in vacuo* to give **4** and the mixture of **5** and **6**.

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